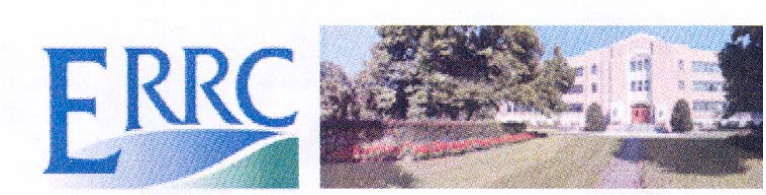

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Title: Inhibitory Activity of Honey Against Foodborne Pathogens as Influenced by the Presence of Hydrogen Peroxide and Level of Antioxidant Power

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Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power

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Abstract

Antimicrobial activity of honey has been attributed to hydrogen peroxide, which is produced by naturally occurring glucose oxidase, and phenolic compounds, although lethality of and inhibition by these and other components against microorganisms vary greatly, depending on the floral source of nectar. This study was undertaken to compare honeys from six floral sources for their inhibitory activity against *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Shigella sonnei*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus*. A disc assay revealed that development of zones of inhibition of growth depends on the type and concentration of honey, as well as the test pathogen. Growth of *B. cereus* was least affected. The inhibition of growth of *S. sonnei*, *L. monocytogenes*, and *S. aureus* in 25% solutions of honeys was reduced by treating solutions with catalase, indicating that hydrogen peroxide contributes to antimicrobial activity. Darker colored honeys were generally more inhibitory than light colored honeys. Darker honeys also contained higher antioxidant power. Since antimicrobial activity of the darker colored test honeys was not eliminated by catalase treatment, non-peroxide components such as antioxidants may contribute to controlling the growth of some foodborne pathogens. The antibacterial properties of honeys containing hydrogen peroxide and characterized by a range of antioxidant power need to be validated using model food systems

Keywords: Honey; Foodborne pathogens; Antioxidant power; Hydrogen peroxide

1. Introduction

Honey has been used as a wound dressing since ancient times. Reports describing inhibition of growth of numerous bacteria of clinical significance have been reviewed (Molan, 1992a,b; Zumla and Lulat, 1989). Healing of skin wounds (Effem, 1988) is

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likely due both to the physical property of osmosis and the antibacterial properties of hydrogen peroxide and non-peroxide components. White et al. (1963) reported that the major antibacterial factor in honey is hydrogen peroxide, which is produced by glucose oxidase originating from hypopharyngeal glands of honey bees. Catalase, which originates from pollen, also occurs in honey. The level of hydrogen peroxide in a given honey is determined by relative levels of glucose oxidase and catalase (Weston, 2000). The higher the glucose oxidase level, the higher the peroxide level and the lower the catalase level, the higher the peroxide level. Differences in antimicrobial activity among honeys from various floral sources may, in part, be a reflection of these variations.

Non-peroxide factors may also contribute to antimicrobial properties of honey (Weston et al., 2000). Components such as lysozyme, phenolic acids, and flavanoids are present in honey (Snowdon and Cliver, 1996). Flavanoids are derived from the propolis, a resinous material collected by bees from gum exudates of trees, and used as an antibacterial agent in hives (Marcucci, 1995). Other phenolic components in nectar (Gil et al., 1995; Ferreres et al., 1996) also have antioxidant activity. Phenolic antioxidants are known to inhibit growth of a wide range of gram-negative and gram-positive bacteria (Davidson, 1993). Frankel et al. (1998) determined the antioxidant capacity of 19 honeys from 14 different floral sources. The highest concentration of antioxidants was 20.3 times that of the lowest. Antioxidant content was positively correlated with both water content and color of honey. Darker color reflects, in part, the content of pigments such as carotenoids and flavanoids, many of which have antioxidant properties.

There is great interest in controlling the growth or eliminating foodborne pathogens using natural antimicrobials. Research that has focused on evaluating light and dark colored honeys from different floral sources for their ability to inhibit the growth of foodborne pathogens has not been reported. Testing this range of honeys for more than one component that may be responsible for inhibitory activity against pathogens has likewise not been reported. The study reported here was undertaken to determine if six honeys from six different floral sources were lethal or inhibited the growth of six foodborne pathogens. The influence of the presence of hydrogen peroxide and level of antioxidant powder on survival and growth of pathogens was assessed.

2. Materials and methods

2.1. Bacterial strains and preparation of inocula

Three strains each of six foodborne pathogens were examined for their ability to survive or grow in media containing six test honeys: *Escherichia coli* O157:H7 (strains F4546 and H1730 from human feces; strain F500, human isolate), *Salmonella typhimurium* DT 104 (antibiotic resistant strains H3380 and H3402) and an antibiotic sensitive strain of *S. typhimurium* (V302-G8430), *Shigella sonnei* (strains F6129, 10304-98, and 10305-98, all isolated from an outbreak of shigellosis associated with raw parsley), *Listeria monocytogenes* (strains G1091, H0222, and V7), coagulase-positive *Staphylococcus aureus* (strains ATTC-13565, ATTC-27664, and ATTC-6538), and toxigenic *Bacillus cereus* (strain B 4ac from pea soup; strain F3802/A84 from pasteurized milk; strain 038-2 from infant formula). All pathogens were grown in tryptic soy broth (TSB, pH 7.3; Difco, Detroit, MI) at 37°C for 24 h before being used as inocula.

2.2. Honeys examined

Six honeys from five floral sources were obtained from Dutch Gold Honey, Lancaster, PA. Honeys were labeled by Dutch Gold Honey as unprocessed (Chinaso buckwheat, Montana buckwheat, and blueberry) and processed (safflower, avocado, and clover), i.e., the particulate material had been removed. Upon receipt, honeys were stored at 21°C in the dark until used. An artificial honey [80% (w/v) sugar], which served as a control, was prepared by dissolving 40 g of fructose, 30 g of glucose, 8 g of maltose, and 2 g of sucrose in 100 ml of distilled water, followed by sterilizing at 121°C for 15 min. This formulation reflects the approximate sugar composition of most honeys (White, 1979), thus, exhibiting osmotic characteristics similar to the six test honeys. The a_w of 25% solutions of honeys was

measured using an Aqualab CX2 (Decagon Devices, Pullman, WA, USA).

Immediately before conducting microbiological assays to determine if survival or growth of pathogens is influenced by honey, all test honeys and the control honey were adjusted to 40°C in a gyrotory waterbath in order to aid pipetting during preparation of diluted honey solutions. Solutions containing 0%, 2.5%, 5%, 10%, 15%, 20%, and 25% (v/v) honeys were prepared in sterile distilled water. These solutions were used to saturate paper disks used in assays to determine zones of inhibition of growth.

2.3. Disc diffusion assay for inhibitory activity

Cultures (0.25 ml) of each test pathogen grown in TSB at 37°C for 24 h were surface spread on tryptic soy agar (TSA, pH 7.2; Difco) in petri plates. Sterile paper discs (5.4 mm diameter; BBL, Cockeysville, MD) were immersed in diluted honey solutions, blotted, and placed on the surface of inoculated TSA. Seven discs were applied to each plate. After incubation at 37°C for 24 h, zones of inhibition surrounding discs were measured with a dial caliper (± 0.1 mm accuracy). The diameter of zones, including the diameter of the disc, were recorded.

2.4. Solution assay for inhibitory activity

Based on results of disc diffusion assays, three strains (*S. sonnei* 10305-98, *L. monocytogenes* V7, and *S. aureus* ATTC 6538) showing sensitivity to test honeys were further studied for survival and growth in 25% honey solutions in 0.1 M potassium phosphate buffer (pH 7.0) treated or not treated with catalase. Five milliliters of honey were combined with 14.8 ml of sterile buffer or 14.8 ml of buffer containing 0.2% bovine liver catalase (Sigma C10, 450 units/mg, St. Louis, MO). After thorough mixing, 0.2 ml of 24-h cultures of test strains were individually added to honey solutions. Inoculated honey solutions were mixed, followed by incubation at 37°C for 24 h. Inoculated honey solutions were surface spread (0.25 ml in quadruplicate and 0.1 ml in duplicate) or diluted in sterile 0.1% peptone water and surface spread (0.1 ml in duplicate) on selective agar media. Colonies formed by *S. sonnei* 10305-98 on xylose lysine desoxycholate agar (XLD, Difco), *L. monocytogenes* V7 on *Listeria* selective agar (LSA, Oxoid, Basingstoke, Hampshire, UK) supplemented with 0.1% colistin methane sulfonate (Sigma), and *S. aureus* ATCC 3568 on TSA supplemented with 10% sodium chloride after incubation for 24 h at 37°C were counted.

2.5. Chemical and physical analyses

Non-heated (control) and heated honeys were assessed subjectively for color, granularity, viscosity, and antioxidant power. Aliquots of honey (ca. 10 ml) were held at 100°C for 8 min. Non-heated and heated honeys were diluted 10-fold in Butterfield's phosphate buffer (0.25 M KH_2PO_4 adjusted to pH 7.2 with NaOH) before color was determined by measuring absorbance at 593 nm and antioxidant power was measured using the ferric reducing/antioxidant power (FRAP) assay, which measures the total antioxidant power (Benzie and Strain, 1999). Samples (50 μl) of each diluted honey were placed in spectrophotometer cuvettes and 1.5 ml of fresh FRAP reagent solution was added, followed by thorough mixing. The reaction was allowed to proceed for 6 min at 21°C to allow full development of blue pigmentation. The absorbance of the reacted solutions was read at 593 nm in a spectrophotometer.

2.6. Statistical analyses

All data presented represent mean values from three replicate experiments. For the zone of inhibition assay, the general linear model (GLM) and Duncan's multiple range test (SAS Institute, Cary, NC) were used, with honey as the class variable (to determine significant differences ($\alpha = 0.05$) in zone diameters between honeys at a given concentration for a given strain) or with concentration as the class variable (to compare differences between concentrations for a given honey within a strain). Differences in populations of pathogens in inoculated, buffered honey solutions were also analyzed by strain and treatment (to determine the effects of catalase on survival or growth) or by strain and type of honey (to compare populations in catalase-treated and control solutions of a given type of honey) using the GLM and Duncan's test.

Pooled data from color and antioxidant power analysis of honeys were scaled, based on the weight of honey in test samples. Since the diluted samples differed noticeably from the undiluted honeys in terms of viscosity and granularity, data were not adjusted for the dilution factor, but are presented as obtained. Values for antioxidant power were converted to FRAP μM equivalents using a standard curve (1000 μM ascorbic acid = 2000 μM FRAP). Data were analyzed for significant differences ($\alpha = 0.05$) between honeys with analysis of variance (ANOVA), for correlation between A_{593} and antioxidant power using Pearson product moment correlation (SigmaStat ver. 2.03, SPSS, Chicago, IL), and non-linear regression (SigmaPlot ver. 5.00, SPSS) for non-heated and heated honeys.

3. Results

3.1. Disc diffusion assay

The disc diffusion assay enabled screening of honeys for inhibition of growth of pathogens as affected by floral source and concentration. The presence and diameter of zones of inhibition was dependent upon both of these experimental parameters as well as by the test pathogen and, to some extent, the strain within each pathogen. For example, the zone of inhibition of growth of *S. typhimurium* H3402 surrounding discs soaked in a 25% solution of avocado honey was significantly larger than those surrounding of other test honeys. More honeys inhibited growth of *S. sonnei* than *E. coli* O157:H7 or *S. typhimurium*. Discs soaked in 25% Chinaso buckwheat, blueberry, avocado, or clover honey solutions caused significantly greater inhibition of *S. sonnei* 10304-98 compared to artificial honey. Growth of *S. sonnei* 10305-98 was significantly more inhibited by Chinaso buckwheat honey than 20% or 25% concentrations of all other honeys.

Among the test pathogens, growth of *B. cereus* was the least affected by honeys. None of the test strains were inhibited by any of the honeys, regardless of concentration. *L. monocytogenes* G10901 and V7 were more sensitive than strain H0222 to honey, with inhibition most evident around discs soaked in the highest concentrations of solutions. Overall, strain V7 was most sensitive to the range of honeys tested. No single honey exhibited exceptional inhibitory activity. Significantly larger zones of inhibition of *S. aureus* strain ATCC 27664 occurred around discs soaked in blueberry or avocado honey, and strain ATCC 6538 around discs soaked in all honeys except avocado.

3.2. Solution assay for survival or growth

One strain each of three pathogens showing sensitivity to several honeys, as determined by the disc diffusion assay, was tested for survival or growth in buffered 25% solutions of honey treated or not treated with catalase. Results are shown in Table 1. The pH of honey solutions ranged from 6.43 to 6.94 and the a_w ranged from 0.976 to 0.983 at the time of inoculation. These conditions would not be expected to substantially affect populations after incubation of honey solutions for 24 h at 37°C. Initial populations of *S. sonnei* 10305-98, *L. monocytogenes* V7, and *S. aureus* ATCC 6538 in inoculated honey solutions were 4.70, 6.07, and 5.82 $\log_{10}$ CFU/ml, respectively. After incubation for 24 h, *S. sonnei* was not detected in artificial honey (control) solution, regardless of treatment with catalase. The population of *L. monocytogenes* was reduced by 4.6 $\log_{10}$ in 25% artificial non-treated honey solution but increased by 1.1 $\log_{10}$ in artificial honey solution treated with catalase. *S. aureus* was not detected in non-treated artificial honey solution and was reduced by 2.4 $\log_{10}$ in artificial catalase-treated solution after 24 h at 37°C. Death of pathogens in artificial honey not treated with catalase is attributed to nutrient deprivation. Viability of *L. monocytogenes* and *S. aureus* was maintained in artificial honey treated with catalase, suggesting utilization of the enzyme as a nutrient source.

A comparison of behavior of pathogens in honey solutions reveals that growth of *S. sonnei* was most inhibited in Chinaso buckwheat honey, regardless of treatment with catalase. Growth of *L. monocytogenes* in the two catalase-treated buckwheat honey solutions was significantly inhibited compared to growth in catalase-treated blueberry, safflower, and clover honey solutions. Of the three test pathogens, *S. aureus* was most sensitive to the range of honeys examined in this study. Compared to initial popula-

Table 1
Populations of foodborne pathogens in 25% honey solutions not treated or treated with catalase, then stored at 37°C for 24 h

Honey solution[1]			Population ($\log_{10}$ CFU/ml of honey solution)[2]		
Variety/treatment	pH	a_w	*S. sonnei* 10305-98	*L. monocytogenes* V7	*S. aureus* ATTC 6538
Control (not treated with catalase)					
Artificial[3]	6.68	0.981	a 0.00 e[4]	b 1.48 c	b 0.00 f
Chinaso buckwheat	6.52	0.981	a 5.95 d	b 6.35 b	b 5.39 c
Montana buckwheat	6.43	0.976	b 6.45 c	b 6.38 b	b 2.20 e
Blueberry	6.57	0.983	a 7.26 a	b 8.05 a	b 3.69 d
Avocado	6.56	0.982	b 7.29 a	b 7.06 ab	b 6.90 a
Safflower	6.62	0.981	b 6.84 b	b 6.58 b	b 6.53 b
Clover	6.69	0.980	a 7.43 a	b 6.94 ab	b 5.51 c
Catalase treated					
Artificial	6.94	0.983	a 0.00 e	a 7.18 d	a 3.47 d
Chinaso buckwheat	6.54	0.978	a 6.45 d	a 7.74 c	a 5.85 bc
Montana buckwheat	6.54	0.982	a 7.21 bc	a 7.80 c	a 6.84 ab
Blueberry	6.62	0.980	a 7.54 a	a 8.95 a	a 5.14 c
Avocado	6.63	0.977	a 7.64 a	a 8.21 bc	a 7.66 a
Safflower	6.68	0.983	a 7.48 ab	a 8.56 ab	a 7.88 a
Clover	6.74	0.981	b 7.07 c	a 8.55 ab	a 7.24 a

[1]Honey solutions (25%) were prepared in 0.05 M potassium phosphate buffer (pH 7.0) (control) or buffer containing 0.2% catalase (treated).

[2]Initial populations of pathogens in honey solutions were 4.70 $\log_{10}$ CFU of *S. sonnei* 10305-98/ml, 6.07 $\log_{10}$ CFU of *L. monocytogenes* V7/ml, and 5.82 $\log_{10}$ CFU of *S. aureus* ATTC 6538/ml. Within control or catalase-treated honeys, and within pathogen, values not followed by the same letter are significantly different ($\alpha = 0.05$). Within each variety of honey (includes control and catalase-treated), and within pathogen, values not preceded by the same letter are significantly different ($\alpha = 0.05$).

[3]80% (w/v) Sugar (40% fructose, 30% glucose, 8% maltose, and 2% sucrose) diluted to 25% (v/v).

[4] < 0.33 CFU/ml.

tions in honey solutions, populations of *S. sonnei* and *L. monocytogenes* increased during the 24-h incubation period, regardless of type of honey or treatment with catalase, whereas counts for *S. aureus*

Table 2
Appearance, absorbance, and antioxidant power of six honeys

Treatment	Variety	Sensory characteristics[1]	Absorbance (593 nm)[2]	FRAP (μM)
Non-heated	Chinaso buckwheat	very dark color, slightly granular, thick, strong aroma	1.02 ab	1131.3 ab
	Montana buckwheat	dark color, granular, very thick, strong aroma	0.56 abc	1009.3 ab
	Blueberry	dark color, very granular, very thick, distinct aroma	0.18 abcd	345.8 abc
	Avocado	medium color, clear, thin, slight aroma	0.09 bcd	141.6 bcd
	Safflower	light color, clear, thin, slight aroma	0.05 d	279.1 cd
	Clover	light color, small granules, thin, slight aroma	0.06 d	139.9 cd
Heated	Chinaso buckwheat	very dark color, clear, thick, strong aroma	1.17 a	1353.5 a
	Montana buckwheat	dark color, clear, very thick, strong aroma	0.71 ab	1182.2 ab
	Blueberry	dark color, clear, very thick, strong aroma	0.23 abc	337.8 abcd
	Avocado	medium color, clear, thin, slight aroma	0.10 bcd	131.7 bcd
	Safflower	light color, clear, thin, slight aroma	0.07 cd	275.5 cd
	Clover	light color, clear, thin, slight aroma	0.07 bcd	120.3 d

[1]Subjective assessment of undiluted honey.

[2]Diluted (10%) solutions were analyzed; values in the same column not followed by the same letter are significantly different ($\alpha = 0.05$).

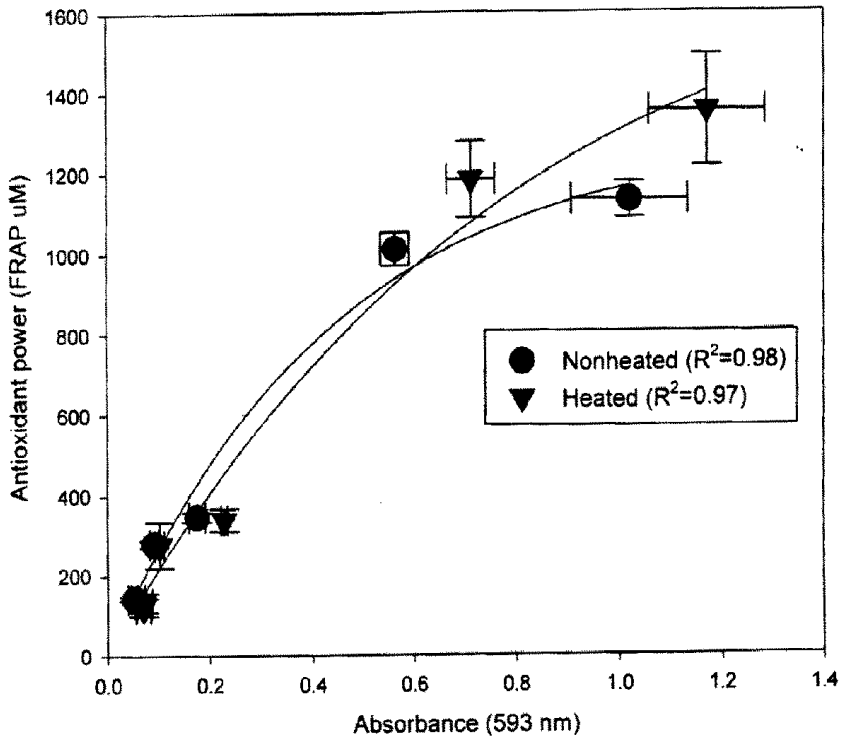

Fig. 1. Relationship between absorbance ($A_{593\ nm}$) and antioxidant power of six 10% honey solutions. Data were pooled from three replicate experiments ($n = 9$); bars indicate standard deviations. Non-linear regression lines indicate exponential rise to maxima: 35.58 μM FRAP (non-heated) and 50.14 μM FRAP (heated) for increasing $A_{593\ nm}$.

decreased in solutions of non-treated Chinaso buckwheat, Montana buckwheat, blueberry, and clover honeys and catalase-treated blueberry honey.

Within each type of honey, populations of *L. monocytogenes* and *S. aureus* were significantly ($\alpha = 0.05$) higher in catalase-treated samples compared to non-treated control samples after the 24-h incubation period. With the exceptions of artificial, Chinaso buckwheat, blueberry, and clover honeys, the same is true for *S. sonnei*.

3.3. Appearance and antioxidant power of honey

Honeys were noticeably different from each other with regard to color, granularity, viscosity, and aroma (Table 2). Heated samples differed from non-heated samples only with regard to granularity, with the heating process serving to dissolve crystallized sugars. There were significant ($\alpha = 0.05$) differences among the honeys in absorbance and antioxidant power, both before and after heat-treatment. There was a significant ($\alpha = 0.05$) correlation between absorbance and antioxidant power, with darker, more opaque honeys having stronger antioxidant power than lighter, clearer honeys. Heat treatment did not significantly alter the absorbance or antioxidant power of any of the six honeys studied. The relationship between absorbance and antioxidant power is illustrated by non-linear regression (Fig. 1). The equation used is of the form $A_{593\ nm} = a(1 - b^{FRAP})$. The values of parameters a and b are 1287.32 and 0.0998 ($R^2 = 0.98$), respectively, for non-heated honeys and 1814.0 and 0.2833 ($R^2 = 0.97$), respectively, for heated honeys.

4. Discussion

Several studies have used well diffusion assays to determine antimicrobial activity of honeys (Molan, 1992a,b). We used a disc diffusion assay with the intent of achieving concentrations of honey on the

inoculated surface of TSA immediately surrounding the disc similar to those in test solutions in which discs were soaked. After incubating TSA plates for 24 h, light to dark brown haloes formed around discs. The intensity of color was correlated with the type and concentration of honey in solutions used to soak discs and generally, but not always, with inhibition of growth of pathogens.

Variability in antibacterial activity of honeys from different floral sources against several species of bacteria known to cause spoilage of foods (Allen et al., 1991) or wound infections (Willix et al., 1992) has been described. In our study, each foodborne pathogen and, in some instances different strains of a given pathogen, exhibited different sensitivities to each of the test honeys. A standard antimicrobial, e.g., phenol, was not used to determine zones of inhibition of pathogens against which inhibitory activity of various concentrations of each honey could be compared. Nevertheless, strains of five of the six pathogens did exhibit sensitivity to one or more honeys at concentrations of 25% or less, indicating that one or more antimicrobial factors were exhibited.

E. coli has been associated with honey bees (Shimanuki and Knox, 1991) but *E. coli* O157:H7 and the other pathogens examined in our study have not been reported to be found in honey. The lack of inhibition of *B. cereus* may reflect a generally higher tolerance of *Bacillus* species to antimicrobials that may be present in honey. *Bacillus* species have been detected in honey (Snowdon and Cliver, 1996) and feces of bee larvae (Gillian and Prest, 1987).

The ability of bacteria to survive in honey varies. Tysset and Durand (1973) reported that *E. coli* survived less than 10 days when inoculated honey was stored at 20°C, whereas *S. typhimurium* survived for 30 days. Another study showed that *S. typhimurium* can survive in honey at 10°C for more than 2 years and *Shigella* can survive for almost 3 months (Tysset and Durand, 1976). In our study, compared to *S. sonnei* and *L. monocytogenes*, *S. aureus* was more adversely affected when inoculated into 25% honey solutions. Substantial reductions in populations of *S. aureus* occurred in Montana buckwheat, blueberry, and Chinaso buckwheat honey solutions not treated with catalase compared to reductions in respective solutions treated with catalase, suggesting that among the three pathogens examined using the liquid assay, *S. aureus* is most sensitive to hydrogen peroxide present in test honeys. Suppression of growth of *S. aureus* in catalase-treated Chinaso buckwheat and blueberry honeys, however, may be attributable to non-peroxide related factors. Catalase-treated solutions of these two unprocessed honeys supported the growth of significantly lower populations of *S. aureus* compared to the three treated processed honeys. Whether growth was inhibited by antimicrobials other than peroxide or perhaps unavailability of nutrients is not known.

The observation that darker colored honeys have higher antioxidant power than light colored honeys confirms findings reported by Frankel et al. (1998). Our study also shows that, although changes in absorbance and antioxidant power related to heat treatment were not significant for any of the test honeys, the overall relationship between color and antioxidant power is influenced by heat. Modeling the relationship between these factors reveals that antioxidant power tends to rise to a maximum as color intensifies, rather than follow a simple linear relationship. For heated honeys, this maximum is approximately 40% higher than for non-heated honeys (1814.0 μM vs. 1287.32 μM FRAP, respectively). This is due primarily to the response of more intensely colored honeys to heat treatment. The mechanism by which heat treatment alters antioxidant power is not fully understood, and implications of the heat alteration for the antioxidant contribution of honeys intended to be used in thermally processed foods on microbiological stability are not known. The complex chemical nature of foods will require that this phenomenon be examined using challenge studies involving foods in which honey may be used for the purpose, in part, to control the growth of specific pathogenic bacteria.

The three unprocessed honeys evaluated in this study were, overall, more inhibitory than processed honeys to growth of five of the six foodborne pathogens tested. The darker, opaque, unprocessed honeys also contained the highest levels of antioxidant power. This correlation would support the notion that antioxidants present in honey contribute to antibacterial activity.

Others have described antimicrobial activities of antioxidants against foodborne pathogenic bacteria

(Ayaz et al., 1980; Klindworth et al., 1979; Robach and Pierson, 1979), yeasts (Eubanks and Beuchat, 1983), and molds (Chang and Branen, 1975), and honey is known to contain flavanoids and other phenolic compounds possessing antioxidant activity (Ferreres et al., 2000). In our study, honeys were treated with sufficient catalase to eliminate hydrogen peroxide. Nevertheless, growth of *S. sonnei*, *L. monocytogenes*, and *S. aureus* in catalase-treated solutions of unprocessed honeys was, overall, retarded compared to growth in processed honeys. This suggests that non-peroxide components in honey do contribute to antibacterial activity but does not support the conclusion drawn by Weston (2000) that non-peroxide antibacterial activity reported to be present in New Zealand's manuka honey (Allen et al., 1991; Weston et al., 1999) should be interpreted as residual hydrogen peroxide activity. Factors in honey contributing to inhibition of growth of foodborne pathogens have clearly not been fully defined. Further work is needed to separate the effects of peroxide and other components in honeys on survival and growth pathogenic and non-pathogenic foodborne microorganisms.

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